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Chemical refining methods effectively mitigate 2-MCPD esters, 3-MCPD esters, and glycidyl esters formation in refined vegetable oils

Sergio B. Oey [a], H.J. van der Fels-Klerx [a], Vincenzo Fogliano [b], Stefan P.J. van Leeuwen [a,*]

[a] *Wageningen Food Safety Research (WFSR), Wageningen University & Research, Akkermaalsbos 2, 6708 WB Wageningen, the Netherlands*
[b] *Department of Food Quality and Design, Wageningen University & Research, P.O. Box 17, 6700 AA Wageningen, the Netherlands*

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ABSTRACT

Esters of 3-monochloro-1,2-propanediol (3-MCPDE), 2-monochloro-1,3-propanediol (2-MCPDE), and glycidyl esters (GE) are processing contaminants that can be found in refined edible fats and oils. Recently, the European Commission has implemented maximum limits for the presence of free and bound 3-MCPDE in vegetable fats and oils and in marine and fish oils. This boosted the necessity of oil producers to develop refining methods to limit the concentration of both 3-MCPDE and GE in their final products. Physical refining may lack the potential to mitigate the formation of 2- and 3-MCPDE. Therefore, in this study, the chemical refining method were explored to provide a viable mitigation strategy aimed at industrial application. Several pilot plant treatments with organic palm oil were performed. The investigated refining methods included a neutralization, a water washing process, reduced deodorization temperature, and a combination of them. The best performing chemical refining treatment achieved a final concentration of 0.42 (−49%), 0.78 (−52%), and 0.99 (−73%) mg/kg for 2-MCPDE, 3-MCPDE, and GE in organic palm oil, respectively. Results thus showed chemical refining has great potential for the simultaneous mitigation of 2-, 3-MCPDE, and GE.

1. Introduction

In recent years, many studies about mitigating the formation of 3-monochloropropane-1,2-diol fatty acid esters (3-MCPDE), and glycidol fatty acid esters (GE) in refined vegetable oils have been conducted. Together with 2-monochloropropane-1,3-diol fatty acid esters (2-MCPDE), 3-MCPDE and GE are potentially carcinogenic compounds which can particularly be abundant in refined palm oil. In 2018, the European Commission has defined a maximum limit (ML) for the concentration of GEs in vegetable fats and oils (European Commission, 2018). In continuation, the European Commission published an Annex (D066084/02) regarding an amendment on the Regulation (EC) 1881/2006 in 2020, in which a ML has been established for the sum of the presence of 3-monochloropropane-1,2-diol (3-MCPD) and 3-MCPDE in vegetable oils, fish oils, and oils from other marine organisms next to the existing ML for GE (European Commission, 2020).

Physical oil refining method in the food industry is widely used. Studies on physical refining methodologies to reduce the formation of 3-MCPDE and GE in vegetable oils have been published, often solely tested in a lab environment, as recently reviewed by Gao et al. (2019) and Oey et al. (2019). Gao et al. (2019) investigated 18 publications about mitigation approaches for 3-MCPDE and concluded that diacylglycerols (DAGs), monoacylglycerols (MAGs), free fatty acids (FFAs), and chlorine can be seen as the precursors for 3-MCPDE, but many papers are still contradictive. Gao et al. (2019) also summarize several potential mitigation strategies including controlling the deodorization temperature, adding chelating agents, changing CPO processing conditions, and many more. However, these potential mitigation strategies are often the results of lab experiments, which has to overcome its own challenges when it is going to be upscaled to a full-scale refining. Oey et al. (2019) primarily shows the lack of data and mitigation strategies for 2-MCPDE, but also highlighted that the majority of the published strategies are often tailored for one of the three process contaminants. Furthermore, physical refining strategies were most successful in mitigating GE, but has less impact on 3-MCPDE. Interestingly, only few papers have reported the occurrence of unbound 3-MCPD and/or unbound glycidol and, at the moment of writing, there are no peer-reviewed publication available regarding mitigation strategies aimed at unbound 2-, 3-MCPD, and glycidol (MacMahon et al., 2013; Zelinková et al., 2006). With the addition of unbound 3-MCPD to the EU regulations, more data is requested to have a better understanding of the relevance of unbound MCPDs in the refined oil products.

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* Corresponding author.
E-mail address: Stefan.vanleeuwen@wur.nl (S.P.J. van Leeuwen).

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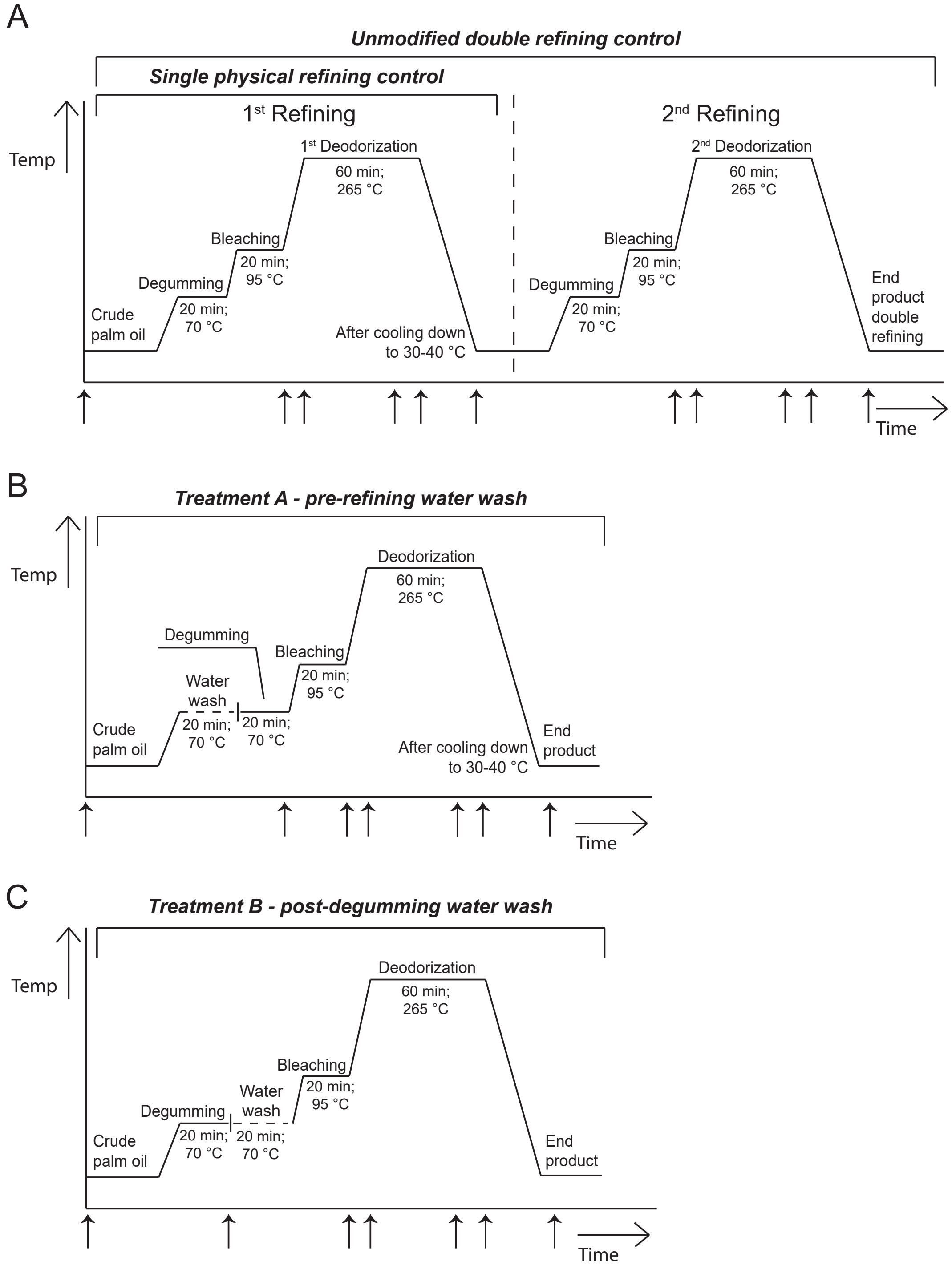

Fig. 1. Schematic depiction of the temperature-time profile of the control treatment (1A), pre-refining water wash (1B), and post-degumming water wash (1C). The vertical arrows underneath the graphs indicate the sampling moments.

The chemical refining route has been investigated infrequently because of increased product losses as oil is being neutralized (e.g. saponification) (Chumsantea et al., 2012; Dijkstra, 2016). Several publications have mentioned the advantages of chemical refining in comparison to physical refining, including the effective removal of gums and FFAs in oil types that cannot be refined well via the physical approach and that chemical refining removes less desired components such as tocopherols, phenols, and sterols. However, compared to physical refining, chemical refining is often related with higher neutral oil loss, larger waste product, and higher chemical usage (Pal, Patra, Sahoo, Bakhara, & Panda, 2015; Zhu et al., 2015; Gotor & Rhazi, 2016).

When extrapolating mitigation methods investigated at the lab-scale, difficulties might be encountered during upscaling to an industrial-scale refining method. Not only is the physical geometry of the lab-scale set-up often very different from a full-scale refinery tower, but other physiochemical attributes such as surface area of the oil or the thermal capacity are different as well after upscaling the method.

According to several proposed formation pathways, the formation of 2- and 3-MCPDE requires a certain chlorine source (Destaillats, Craft, Sandoz, et al., 2012; Šmidrkal et al., 2016; Yao et al., 2019). It can be expected that small amounts of chlorine are present in the crude palm oil. Zhao et al. (2016) showed the potential of various organic and

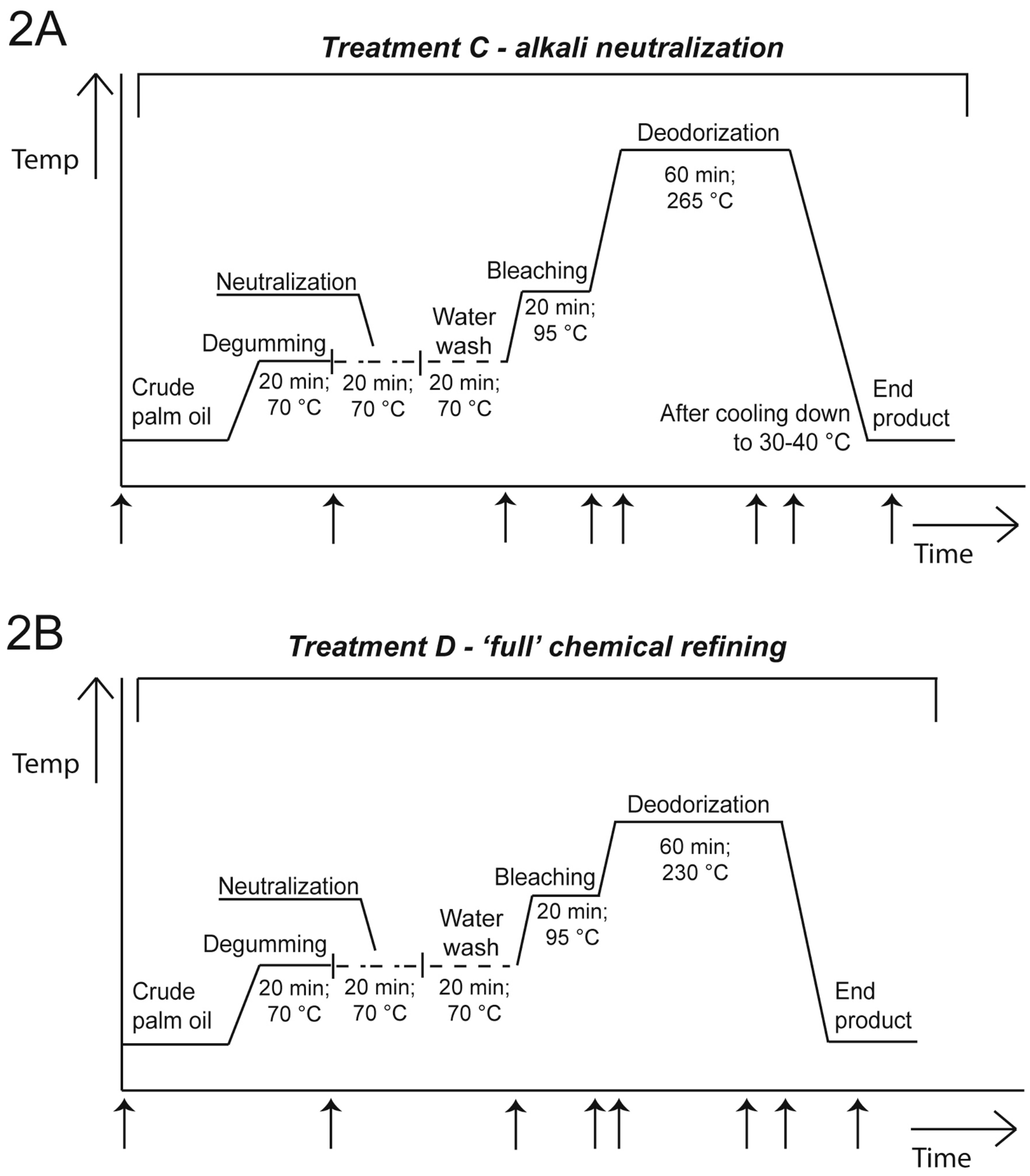

Fig. 2. Schematic depiction of the temperature-time profile of Treatment C - alkali neutralization treatment with a water wash (2A) and Treatment C - 'full' chemical refining treatment (2B) which has a lower deodorization temperature. The vertical arrows underneath the graphs indicate the sampling moments.

inorganic chlorides, such as lindane, KCl, NaCl, and $FeCl_3$, reacting with monoglycerides to form 3-MCPDE and GE. Furthermore, Svejkovská et al. (2006) have reported that other glyceride compounds, such as 1-monopalmitin or tripalmitin may also be seen as precursors for 3-MCPDE. Based on that knowledge, removal of the inorganic chlorine by washing the crude palm oil with deionized water prior to refining can be one of many plausible mitigation strategies. In addition, only a limited number of published mitigation strategies have included 2-MCPDE; most of them focused on 3-MCPDE.

Lampen (2022) have summarized the toxicology of 2-MCPDE and concluded that more toxicological data about 2-MCPDE is required to gain better understanding of its toxicity, but also reported that 2-MCPDE's toxicity may trigger different mode of action *in vivo* than 3-MCPDE. Furthermore, Buhrke et al. (2015) reported that upon hydrolysis in the gut, free 2-MCPD is cytotoxic at concentrations above 1 mM and that the used Caco-2 cells showed reduced cellular viability when subjected to 2- and 3-MCPDE concentrations above 10 μM. Finally, 2-MCPDE toxicity in rat kidney, liver, and heart has also previously been reported (Frenzel et al., 2018; Schultrich et al., 2017). Therefore, mitigation strategies should not neglect 2-MCPDE as its toxicity has been shown in several studies.

Chemical refining involves a neutralization step of the oil with a base (usually a lye), followed by the removal of FFAs by water washing. Whenever unbound or inorganic chlorine is still present after the neutralization process, it can be expected that they will be removed together with the FFAs during the water washing process. The potential of the neutralization process in the development of a good mitigation strategy has been noted. Ramli et al. (2011) succeeded to lower the 3-MCPDE concentration from 2.2 to 1.4 mg/kg with 0.2% CaO, and Freudenstein et al. (2013) achieved a similar end concentration of 1.1

Table 1
Experimental conditions of the four chemical refining treatments (Treatment A - D) and one control treatment (a double physical refining) which were refined in the pilot plant.

	Water wash of CPO	Degumming	Neutralization	Water wash	Bleaching	Deodorization
Control Treatment (single & double physical refining)	–	20 min., 70 °C, 0.5 % citric acid (from a 25 % citric acid solution)	–	–	20 min., 95 °C, 1.0 % Pure-Flo® B80, 0.1 % Norit®, 0.2 % Dicalite® 478	60 min., 265 °C, strip-steam, 3 mbar vacuum, rapid cooling down after 60 min.
Treatment A – pre-refining water wash	20 min., 70 °C, 10 % DI-water (water pre-heated to 70 °C)	20 min., 70 °C, 0.5 % citric acid (from a 25 % citric acid solution)	–	–	20 min., 95 °C, 1.0 % Pure-Flo® B80, 0.1 % Norit®, 0.2 % Dicalite® 478	60 min., 265 °C, strip-steam, 3 mbar vacuum, rapid cooling down after 60 min.
Treatment B – post-degumming water wash	–	20 min., 70 °C, 0.5 % citric acid (from a 25 % citric acid solution)	–	20 min., 70 °C, 10 % DI-water (water pre-heated to 70 °C)	20 min., 95 °C, 1.0 % Pure-Flo® B80, 0.1 % Norit®, 0.2 % Dicalite® 478	60 min., 265 °C, strip-steam, 3 mbar vacuum, rapid cooling down after 60 min.
Treatment C – alkali neutralization	–	20 min., 70 °C, 0.5 % citric acid (from a 25 % citric acid solution)	20 min., 70 °C, 2 L of 33 % NaOH solution	20 min., 70 °C, 10 % DI-water (water pre-heated to 70 °C)	20 min., 95 °C, 1.0 % Pure-Flo® B80, 0.1 % Norit®, 0.2 % Dicalite® 478	60 min., 265 °C, strip-steam, 3 mbar vacuum, rapid cooling down after 60 min.
Treatment D – 'full' chemical refining	–	20 min., 70 °C, 0.5 % citric acid (from a 25 % citric acid solution)	20 min., 70 °C, 2 L of 33 % NaOH solution	20 min., 70 °C, 10 % DI-water (water pre-heated to 70 °C)	20 min., 95 °C, 1.0 % Pure-Flo® B80, 0.1 % Norit®, 0.2 % Dicalite® 478	60 min., 230 °C, strip-steam, 3 mbar vacuum, rapid cooling down after 60 min.

mg/kg 3-MCPDE with 1 mmol/kg $NaHCO_3$. A water washing step can also be implemented prior to the actual start of the oil refining. Crude oil can be mixed with deionized water to remove water soluble precursors. Ramli et al. (2011) and Zulkurnain et al. (2013) reported final 3-MCPDE concentrations of 0.75 mg/kg and 0.2 mg/kg, respectively, with a pre-refining water wash process. In a chemical refining, the neutralization of the acid during the degumming process is a key factor, together with either a pre-refining or post-neutralization water wash. As most of the FFAs are saponified during neutralization and removed during the post-neutralization water wash, there is no need for high-temperature steam distillation during the deodorization phase. Hrncirik and van Duijn (2011) used neutralization and a 5-hour deodorization process at 180 °C and observed a minimal reduction in 3-MCPDE from 4.8 to 4.1 mg/kg and a notably low GE concentration of 0.4 mg/kg in comparison of their reference conditions (180 °C for 1 h). Altogether, only few studies explored mitigation strategies based on chemical refining, leaving room for improvement and further explorations. None of those studies have significantly explored the potential benefits of combining multiple strategies. Combining several mitigation strategies can result in a good all-round chemical refining mitigation strategy which addresses all the contaminants (2-, 3-MCPDE, and GE) at once. The difficulties lie in the fact that 2- and 3-MCPDE have different mechanism of formation than GE and thus requires a different mitigation approach (Cheng et al., 2017; Destaillats, Craft, Sandoz, et al., 2012; Šmidrkal et al., 2016; Yao et al., 2019). Furthermore, 2- and 3-MCPDE are less thermolabile than GE, therefore a strategy to prevent their formation instead of removing them with a heat treatment, like a deodorization process, might be more successful (Shimizu et al., 2013).

The aim of this study was to investigate the effectiveness of various chemical refining strategies for the mitigating of the formation of 2-, 3-MCPDE, and GE during vegetable oil refining at the industrial scale. Several treatments including water washing were conducted to explore the crucial steps of chemical oil refining.

2. Materials & methods

All materials used for the pilot plant treatments were of food grade quality. The organic crude palm oil (CPO) from Ivory Coast were supplied by Spack B.V. (Nieuwe-Tonge, The Netherlands) and were certificied as organic by Skal Biocontrole (NL-BIO-01) on the basis of Article 29 (1) of Regulation (EC) No. 834/2007 and Regulation (EC) No. 889/2008. Pure-Flo® B80 natural bleaching earth was purchased from Oil-Dri (Ripley, Mississippi, USA). Citric acid, Dicalite® Perlite 478 filter aid, and the sodium hydroxide were purchased from Univar Solutions (Rotterdam, The Netherlands). Norit® SA 4 PAH-HF activated carbon was purchased from Cabot Norit Nederlands B.V. (Amersfoort, The Netherlands). One hundred kg of organic CPO was used as starting material for each pilot plant treatment. Separately, 30 kg of the same organic CPO was used to pre-flush the pilot plant prior to each treatment. 0.5% w/w-oil of a 25% citric acid solution was used during the degumming process. 10 % w/w of pre-heated reverse osmosis (RO) water and 2L sodium hydroxide (33% w/v solution) was used when the treatments required them. Bleaching process was applied for all treatments. This was performed with 1.0% w/w Pure-Flo® B80 natural bleaching earth. A filter bag made from polyester (Eaton CLEARGAF™ polyester needlefelt) was used for the filtration of the oil prior to deodorization. Detailed explanation of the pilot plant, stationed in an oil refinery in Zaandam, and a schematic illustration are described previously (Oey et al., 2020). In essence, the pilot plant, with a capacity of 100 kg oil, consists of a treatment tank, a filtration set-up, and a deodorization tank. Both tanks are equipped with electric heating elements, but the deodorization tank is also equipped with strip-steam capabilities and vacuum can be applied to it. Prior to usage, the pilot plant has been extensively tested by our project partner SRC B.V., validating its performance, in the oil refining process.

2.1. Experimental design

The experiment covered the effects of several treatments, including thermal treatment, water washing, and oil neutralization prior to deodorization, on the formation of the 2-, 3-MCPDE, and GE. The control and the four treatments were all conducted in a pilot plant. Schematic overviews of the temperature–time profile of the control and all treatments are presented in Figs. 1 and 2. Details of the experimental conditions are reported in Table 1. The control (Fig. 1A) was designed as two individual physical refining treatments, performed consecutively. This allows for the other treatments to be compared against a normal single physical refining, which is commonly used for the refining of CPO, and against a worst-case scenario in which the refined palm oil was processed for the second time – using similar conditions as the first refining. The four treatments studied in this experiment are: a pre-refining water wash treatment (Treatment A, Fig. 1B), a post-degumming water wash treatment (Treatment B, Fig. 1C), alkali neutralization of the oil with sodium hydroxide after the degumming

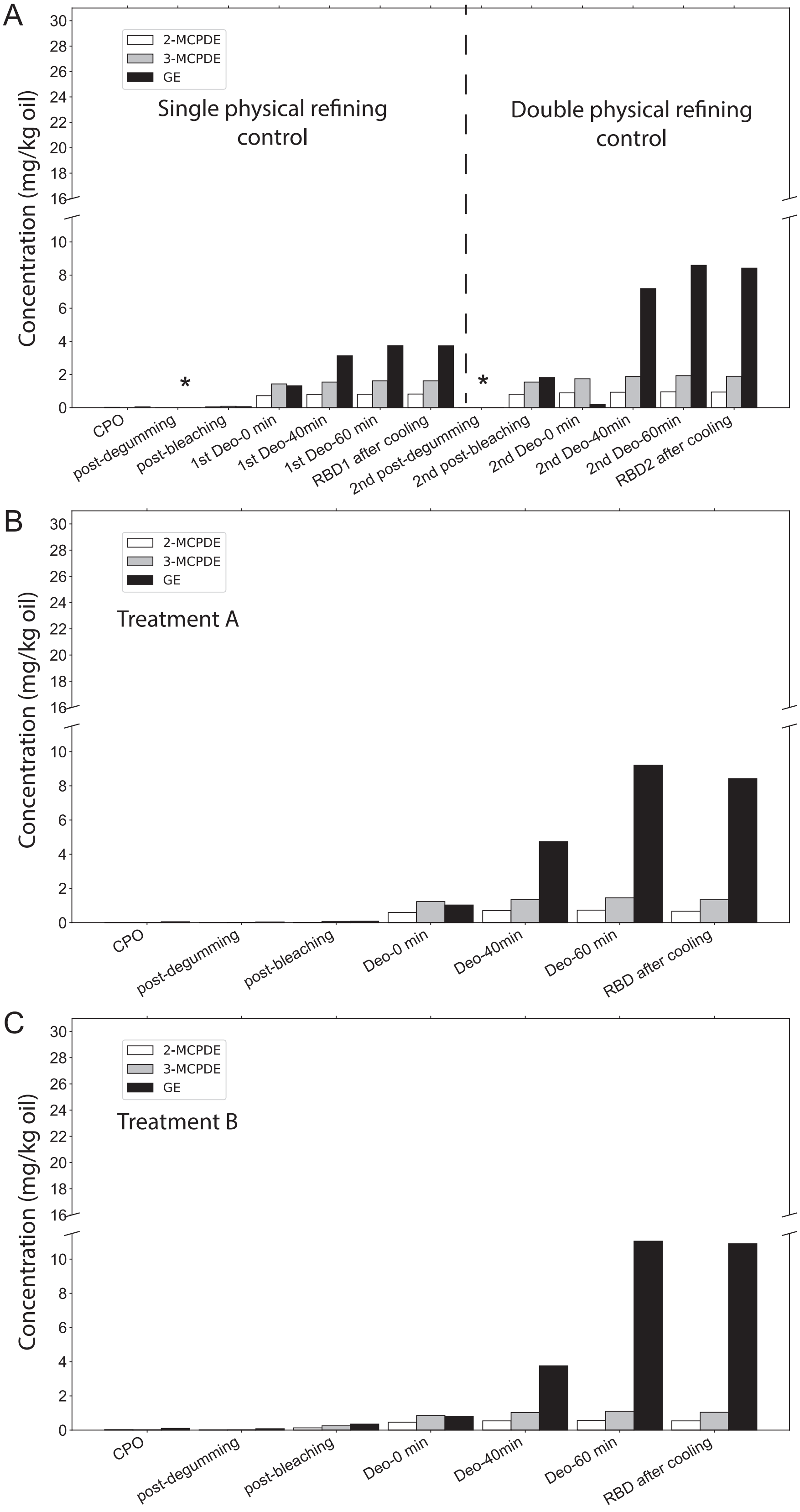

Fig. 3. Results of the two-part control treatment (3A), pre-refining water wash (3B), and the post-degumming water wash (3C). The vertical dashed bar in 3A shows the two separate refining processes that were used as single physical refining control and a 'worst-case scenario' control of an unmodified double refining. The asterisk (*) shows samples that were not collected during refining.

process plus a water wash thereafter (Treatment C, Fig. 2A), and finally a 'full' chemical refining including neutralization, post-degumming water wash, and a lower deodorization temperature which is only required to sterilize the oil (Treatment D, Fig. 2B). These four treatments were selected so that the effect of each individual chemical refining steps on the formation of 2-, 3-MCPDE, and GE can be isolated. This allows for better observation and comparison.

2.2. Sample collection

Sample collection during each treatment was performed manually at the pilot plant. The pilot plant allows for on-line sample collection at any given moment during a refining process. The moment of sample collection during the control and the four experimental treatments are indicated with black arrows in Fig. 1 and Fig. 2. Each of the collected sample was approximately 300 mL in size and were stored in the freezer at −18 °C prior to analysis. The sample that was indicated with 'DEO-0 min' was taken at the beginning of the deodorization process when the oil had reached the set temperature of 265 °C. The 'DEO-40 min' and the 'DEO-60 min' samples were taken after 40 and 60 min of deodorization.

2.3. Analytical determination

The analyses of 2-, 3-MCPDE, and GE in all oil samples were performed with an in-house validated method, at Wageningen Food Safety Research, according the Dutch NEN 7777 standard. Detailed materials and method used for the in-house validation and analyses of 2-, 3-MCPDE, and GE are previously described (Oey et al., 2020). The core concept of this method was based on the AOCS Official Method Cd 29a-13. We expanded the selectivity of this method by introducing a third penta-deuterated internal standard to quantify 2-MCPDE and by performing additional multiple-reaction-monitoring (MRM) measurements in the MS for the phenyl boronic acid (PBA) derivates of 2-, 3-MCPDE, and GE. Essentially, GE was first converted into primarily 3-monobromopropane-1,2-diol esters (3-MBPDE). After the conversion, a mild acid-mediated transesterification of the 2-, 3-MCPDE, and 3-MBPDE was performed. The samples were then cleaned-up using liquid–liquid extraction methods with heptane and, finally, a derivatization step was performed with PBA.

The samples were analyzed using an Agilent 7010B Triple Quadrupole GC–MS/MS system (Agilent, USA). Separation occurred in an Agilent DB-35MS UI GC column (30 m × 0.250 mm × 0.25 μm) (Agilent, USA). A nine-point calibration line in a blank extra virgin olive oil matrix was used for the quantification, including a blank calibration point. The highest calibration point was equivalent to 0.62 μg unbound 3-MCPD, 0.62 μg unbound 2-MCPD, and 0.78 μg unbound glycidol. The results of the in-house validation can be found in the supplemental materials. The average accuracy (i.e. being able to detect the true concentration) for 2-, 3-MCPDE, and GE were determined during the validation of the method, as well as the linearity, limit of quantification (LOQ), and limit of detection (LOD). The method was linear for the used calibration range. The average accuracies were 99%, 98%, and 105%, respectively, for 2-, 3-MCPDE, and GE. The LOQ for 2-, 3-MCPDE, and GE were 0.07, 0.10, and 0.07 mg/kg, respectively. The LOD for 2-, 3-MCPDE, and GE were 0.04, 0.05, and 0.03 mg/kg, respectively. The repeatability and reproducibility of the method were determined by analyzing eight different oil samples in duplicates with varying concentration between the LOD and 3.5 mg/kg. The relative standard deviations (RSD) for the repeatability of 2-MCPDE, 3-MCPDE, and GE are 3%, 2%, and 4%, showing good precision (i.e. has a low amount of spread in the results). Furthermore, the real-life performance of this method has been proven and guaranteed by participating with multiple proficiency tests organized by FAPAS and the European Reference Laboratory for Process Contaminants with satisfactory Z-scores. The extensive in-house validation and the proficiency tests showed that the method is fit for purpose and provides accurate results.

2.4. Physicochemical quality parameters of the oil samples

The water content in the individual oil samples were determined by coulometric Karl Fischer titration. A Metrohm 917 coulometer was used with Hydranal™-coulomat CG as the catholyte solution and Hydranal™-coulomat oil as the anolyte solution. In this study, the water content in the oil samples are expressed in percentages. The FFA concentration, expressed as '% oleic', and the color of the oil were analyzed as well. The FFA was determined by potentiometric titration using sodium hydroxide. Oil color determination was performed manually using a visual colorimeter and a 5¼-inch cuvette on the Lovibond® scale (Red, Yellow, Blue, and Neutral color scale).

3. Results & discussion

3.1. Single & double physical refining control

Results of the control treatment are shown in Fig. 3A. The single physical refining control, which is the first half of the control treatment, resulted in a GE concentration in the end product of 3.73 mg/kg, whereas the concentrations of 2-MCPDE and 3-MCPDE were 0.82 and 1.62 mg/kg, respectively. However, by the end of the second refining step GE concentration was elevated to 8.42 mg/kg. After the formation of 2- and 3-MCPDE in the single refining control, the levels of these two compounds remained stable throughout the entire second physical refining. As a side note, our analytical method has been thoroughly validated in-house and the results' variances caused by the analytical method have been deemed very low. A complete set of the water content results of this control treatment and the other four treatments are presented in Appendix A (Figs. 5–9). Results of the FFA and oil color can be found in the Supplementary material section, Table S1 – S5.

The control treatment was designed to obtain results of both a single physical refining, which is commonly used in the oil industry, and a double physical refining without any modifications. The unmodified double physical refining method does not provide any practical benefits, but it shows the effect of a worst-case scenario in which a refined oil would be subjected twice to high deodorization temperatures.

The observed stability of 2- and 3-MCPDE during the second refining procedure is in line with the proposed mechanisms of the formation of 2- and 3-MCPDE. The formation of 2- and 3-MCPDE requires a chlorine source such as sodium chloride or hydrochloric acid that can be incorporated into a MAGs and/or DAGs via multiple pathways which involves nucleophilic substitution and the formation of an intermediate (Destaillats, Craft, Sandoz, et al., 2012; Hamlet et al., 2011; Rahn & Yaylayan, 2011; Šmidrkal et al., 2016). Once the chlorine atom has been incorporated, it forms a stable covalent bond. Ermacora and Hrncirik (2014) showed that the formation of 2- and 3-MCPDE is positively correlated with the concentration of available chlorine in the oil. When the available chlorine sources were depleted during the first refining in our control, no additional formation of 2- and 3-MCPDE occurred during the second refining. This is in line with earlier observations in another study on physical refining of CPO (Oey et al., 2020).

Interestingly, a drop in the GE concentration after the second bleaching process was observed. GE is known to be less chemically stable than 2- and 3-MCPDE due to its epoxide. Cheng et al., (2020) showed that GEs can indeed be degraded via an acid-induced ring-opening reaction. Furthermore, Shimizu et al., (2012) previously has reported the usage of activated bleaching earth to eliminate GE. They have reported that glycidyl palmitate was transformed into glycerol monopalmitate, glycerol palmitate oleate, and glycerol dipalmitate rather than absorption of GE into the bleaching earth. As acid and bleaching earth was re-introduced to the oil for the second time during the secondary refining process in our control experiment, this change of pH in combination with the bleaching earth might have degraded GE. The increase of GE during the second deodorization is expected due to oil degradation as it is being exposed to high temperature for a second time. Triacylglycerols (TAGs) can degrade into DAGs or MAGs which are precursors for MCPDEs.

3.2. Treatment A – Pre-refining water washing

The first treatment (Treatment A) examined the effect of a pre-refining wash of the CPO with reverse osmosis (RO) water. Results are reported in Fig. 3B. As observed in the single physical refining control, 2-, 3-MCPDE, and GE were formed after the bleaching process. The concentration of 2- and 3-MCPDE started to increase at the beginning of the deodorization process and showed a minor increase in concentration for the remaining duration of the deodorization process. The final concentration of 2- and 3-MCPDE were 0.67 and 1.34 mg/kg, respectively (see Fig. 3B). The concentration of 2-MCPDE were decreased by 18% and 3-MCPDE was decreased by 17% in comparison to the single physical refining control (Fig. 3A).

The levels of GE increased during the deodorization process. After 40 min of deodorization during the secondary physical refining, GE reached a concentration of 8.42 mg/kg. In this treatment, the levels of GE were higher than in the control treatment (Fig. 3A). The exact cause of this increase in GE concentration remains inconclusive, but some speculations can be made. Temperature and duration can be ruled out as the source of the large increase, because those refining parameters are the same as in the physical refining control. Having said that, the water wash process may have caused the increase in GE concentration by hydrolyzing the TAGs into DAGs. Silva et al. (2019) performed a series of experiments in which bleached palm oil (BPO) was washed with different solvents. When BPO was washed a single time with water, they observed a 21% increase in the GE concentration, which is less than what was observed at the end of Treatment A. Furthermore, DAGs concentration was approximately increased by 72%. Previous studies have indicated that GE can be formed from both MAGs and DAGs without the need of a chlorine source via intramolecular rearrangement (Cheng et al., 2016; Cheng et al., 2017; Destaillats, Craft, Dubois, et al., 2012a). Increased concentrations of MAGs or DAGs might therefore lead to higher concentrations of GE.

The relatively high water content of 0.46% (see Fig. 6 in Appendix A) in the ‘post-degumming’ sample was expected because the separation of water and oil was done only by gravitational separation as the pilot plant did not have a centrifuge to efficiently remove the water. After the ‘post-bleaching’ sample, the water content decreased to below 0.1%. It can be expected that most of the water has evaporated by the end of the bleaching process as the oil temperature was set at 95 °C. However, it is possible that this small percentage of water could have indirectly caused the large increase in GE concentration. The residual water might have unfavorably altered the pH or have reacted with the TAGs, acid and/or bleaching earth. An increase of the presence of MAGs and DAGs due to hydrolysis is plausible and cannot be ruled out.

Recently, Ramli et al. (2020) washed 900 Tonne (900.000 kg) of CPO at the palm oil mill using 5–10% hot, softened water (90–95 °C, total chlorine of 5 mg/kg). Two refineries have processed the oil in almost similar manner to our double physical refining control except for the left-out secondary degumming step and the lower second deodorization temperature. The details of the refining conditions were unfortunately not reported. After a single refining process without post-refining processing, the washed CPO sample had a 2-, 3-MCPDE, and GE concentration of 0.49 ± 0.12, 1.37 ± 0.33, and 3.84 ± 0.42 mg/kg, respectively (Ramli et al., 2020). With post-refining bleaching and deodorization, they found a final 3-MCPDE and GE concentrations around 1.4 and 1.2 mg/kg, respectively. Post-refining 2-MCPDE concentration was not reported. The 3-MCPDE concentrations observed by Ramli et al. (2020) were similar to the concentration in our experimental treatment with pre-refining water washing. However, their GE concentrations, with and without post-refining processing, were both lower than those concentrations in our treatment.

3.3. Treatment B – Post-degumming water wash

The second treatment (Treatment B) examined the effect of a water wash treatment performed after a degumming process. More specific, the wash treatment occurred between the degumming process and the bleaching process. The results of this experimental treatment are shown in Fig. 3C. Similar to the pre-refining water washing, the formation of 2-, 3-MCPDE, and GE started after the bleaching process. Again, using the post-degumming water wash, the majority of the 2- and 3-MCPDE were formed during the pre-heating stage prior to the start of the deodorization process. However, in this case (Treatment B) the final concentrations of 2- and 3-MCPDE (0.54 and 1.04 mg/kg, respectively) were, respectively, 19% and 22% lower than the final concentrations observed in Treatment A. Compared to the single physical refining control treatment, this treatment resulted in a reduction of 34% for the 2-MCPDE concentration and 36% for the 3-MCPDE concentration. A wash process after the degumming process resulted in the removal of citric acid that has been used during the degumming step. According to Destaillats, Craft, Sandoz, et al. (2012), acidic environments promote the formation of 3-MCPDE. A wash process beneficially reduces the potential catalytic role of citric acid or any other acids in the formation of 2-, 3-MCPDE, and GE. Therefore, the observed results were in line with the prior expectations that this treatment (Treatment B) results in a lower 2- and 3-MCPDE concentration as compared to the single physical refining control treatment and the pre-refining water wash treatment (Treatment A).

Several similarities and differences were observed comparing the results of this post-degumming water wash with the control treatment. As observed in the control treatment, 2- and 3-MCPDE were formed prior to the start of the deodorization step. Once 2- and 3-MCPDE have been formed, their concentrations remained stable throughout the entire deodorization process. The results were different for the concentration of GE: its formation started during the deodorization process but GE concentration in this treatment rose to 11.05 mg/kg after 60 min of deodorization and the final oil sample after the cool down had a concentration of 10.90 mg/kg. This final GE concentration was higher than the GE concentrations of both the single and double physical refining controls (3.73 and 8.42 mg/kg, respectively). The increased GE concentration in Treatment B might share a similar cause as what was discussed previously in Treatment A. Water that was still present in the oil after the wash process might indirectly promote the hydrolysis of TAGs into MAGs and DAGs and. Furthermore, compared to Treatment A, Treatment B showed a greater GE increase. This can be explained by the deliberate increase of oil temperature right after the washing process in Treatment B to accommodate the bleaching condition. This higher oil temperature after a wash treatment may lead to an increase of reactivity resulting in more MAGs and DAGs formation.

Recently, Hew et al. (2021) optimized a modified refining process in laboratory-scale using a process similar to our post-degumming water washing treatment (Treatment B). The main differences are the volume of the CPO, the adoption of phosphoric acid instead of citric acid, the usage of less water during the water degumming or in our case the post-degumming water wash process (1.0% instead of 10%), and a slightly different temperature–time profile of the deodorization condition (90 min at 250 °C instead of 60 min at 265 °C). With this refining method, Hew et al. (2021) achieved a final 3-MCPDE concentration of 0.71 mg/kg which is slightly lower than ours at the end of this treatment (1.04 mg/kg, Treatment B). Interestingly, their GE concentration of 0.32 mg/kg is lower than at the end of our Treatment B (10.90 mg/kg). Unfortunately, it is almost impossible to compare the quality of the CPO used by Hew et al. (2021) and our CPO. If they have used a higher quality CPO, it might explain why the observed concentrations in their experiments are lower than ours. Furthermore, the differences in the physical experimental set-up makes a direct comparison very challenging. Nonetheless, our results and the results of Hew et al. (2021) indicate the potential of this treatment to mitigate 2-, 3-MCPDE, and GE.

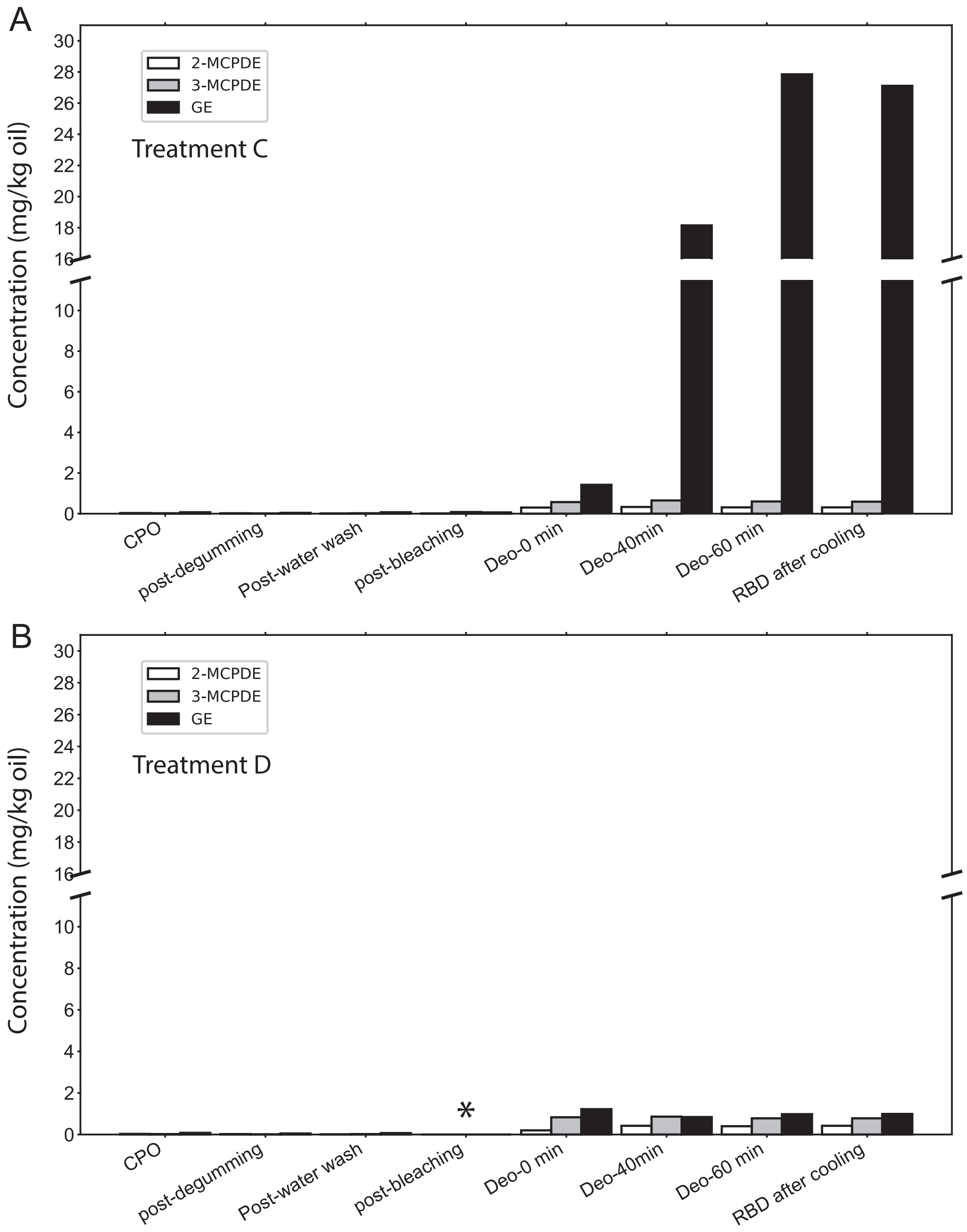

Fig. 4. Results of Treatment C – alkali neutralization (4A) and Treatment D – 'full' chemical refining (4B). The only difference between these two treatments is the lower deodorization temperature in Treatment D (230 instead of 265 °C). The asterisk (*) shows samples that were not collected during refining.

3.4. *Treatment C – Alkali neutralization*

The key factor differentiating this alkali neutralization treatment from the other investigated treatments is the chemical neutralization process. Therefore, the experimental design choice was selected to exclusively monitor the effect of the alkaline neutralization with sodium hydroxide. The addition of sodium hydroxide is useful not only to neutralize the citric acid used for the degumming, but also to remove the majority of the FFAs by turning them into soap stock. As the FFAs are no longer present during the deodorization process, lower deodorization temperatures can be use during chemical refining. Whereas in principle, the deodorization temperature can be lowered after alkali neutralization, the deodorization temperature during this treatment was kept unchanged for the sake of equal comparison with the control and especially with the post-degumming washing treatment (Treatment B).

Looking at the results reported in Fig. 4A, the final concentrations of 2- and 3-MCPDE was 0.31 and 0.59 mg/kg, respectively. This resulted in a 62% and 64% reduction for the 2- and 3-MCPDE, respectively, compared to the single physical refining control. Furthermore, the final 2- and 3-MCPDE concentrations of this neutralization treatment were 43% lower compared to the results of the post-degumming washing process. The main difference between this treatment and Treatment B is the addition of sodium hydroxide in this treatment. With this design we were able to isolate the effect of adding a lye to neutralize the oil. As reported by Šmidrkal et al. (2016), 2- and 3-MCPDE formation is favorable in an acidic environment. The added sodium hydroxide neutralizes the pH of the oil. This results in an inhibition of 2- and 3-MCPDE formation due to the less favorable condition.

The three contaminants (2-, 3-MCPDE, and GE) are formed before the start of the deodorization process. This is similar to what has been observed in the control and previous two treatments. These results showed that the creation of a basic environment is beneficial for the reduction of the formation of 2- and 3-MCPDE. The post-degumming water washing treatment (Treatment B) was able to slightly reduce the 2- and 3-MCPDE formation, however the effect is minimal when compared to the effect of sodium hydroxide in this treatment (Treatment C).

The final GE concentration in this treatment was 29.4 mg/kg, making it the highest observable GE concentration in the experimental treatments of our study. Furthermore, the water content in the ‘post-degumming’ sample was 2.46% which was also the highest among all other samples. Based on the physical–chemical properties of FFAs, it is possible that it might have created an emulsion. This is especially the case when FFAs reacts with a lye and create a soap stock via a reaction called saponification (Chew et al., 2017; Dumont & Narine, 2008) This might explain the relatively high water content which could not effectively be removed due to limitations of the experimental set-up. The water content in the final oil sample of this treatment was 0.04%. This particular refining condition, i.e. addition of sodium hydroxide and high deodorization temperatures, could also have lead to higher formation of MAGs and/or DAGs via hydrolysis of TAGs. Cheng et al., (2016) have reported that the combination of temperatures over 200 °C and the presence of MAGs and DAGs would lead to an increase in GE concentration. However, an extremely high amount of MAGs and DAGs must be present to explain our measured GE concentration in treatment C. According to Zelinková et al. (2017), analytical artefacts leading to biased GE results might be introduced when large amounts of MAGs are present in food samples. These MAG in food samples are often emulsifiers that has artificially been added into the food products. As our samples consists of pure palm oil, we do not expect extreme concentrations of MAGs that could have caused such analytical artefacts. Additionally, our analytical method includes a liquid–liquid extraction step where FFAs are removed post-transesterification. Large amount of FFAs from MAGs would, therefore, be removed during the sample preparation. Therefore, with a slight note of cautiousness, we consider the observed GE concentration to be an undesired effect from this treatment. Further investigation is required to confirm this adverse effect.

As a side note, the ‘post-degumming’ sample was taken right after the addition of the sodium hydroxide when the degumming process had finished. The soap formation, which commonly occurred after the addition of sodium hydroxide, caused two challenges. First, the separation of water and oil took twice as long (>1.5 hr) in comparison to what was needed during Treatments A and B. As the formed soap stock acted as surfactant, an emulsion was easily created. This emulsion was more stable than when only oil and water would have been mixed, as was the case in Treatment A and B. Therefore, it was more difficult to achieve a good water and oil separation. Beside this, the soap phase might help with the removal of polar chlorine containing molecules, metallic-, and inorganic chlorides.

The combination of observations from Treatments A and B with the observation from the current treatment leads to hypothesis that sodium hydroxide, the relatively high water content after the degumming process, possible increase in DAGs and MAGs formation, and the unadjusted high deodorization temperatures resulted in abundant formation of GE. Secondly, the usage of lye to neutralize and remove FFAs is known for its increased oil losses. The compromise is incorporated in our main aim which is the development of a practical way to simultaneously reduce the amount of 2-, 3-MCPDE, and GE. Especially because the European Commission has implemented new MLs for unbound 3-MCPD and bound 3-MCPDE. Successive research can focus on making this chemical refining method more efficient.

3.5. *Treatment D – Chemical refining*

The results of Treatment D are shown in Fig. 4B. Similar to the previous three experimental treatments and the control, 2- and 3-MCPDE started to form after the bleaching process, prior to the start of the deodorization. Continuing the mild deodorization process (230 °C, 1 h), GE concentration stabilizes at 0.99 mg/kg, while the final 2- and 3-MCPDE concentration reached 0.42 and 0.78 mg/kg, respectively. Lower deodorization temperature can be utilized in chemical refining as the majority of the FFAs were already removed during the neutralization process. Compared to the single physical refining control, 2- and 3-MCPDE were reduced by 49% and 52%, respectively, while GE was reduced by 73%. Upon comparison with other physical refining methods, one can notice that our GE concentration might not be as low as the reported GE concentrations in physical refined oil (Sim et al., 2020). However, our primary goal was to simultaneously mitigate the concentration of 2-, 3-MCPDE, and GE. Furthermore, the main advantage of our method and experiment is the scale of experimentation and that all our experiments have been performed in a pilot plant. With sample sizes of 100 kg for each treatment, our pilot plant offers better simulation of the full-scale industrial refining. Additionally, upscaling a lab-scale experiment is no easy feat as demonstrated by Sim et al. (2020). With our pilot plant experiments, we were able to simulate an on-line degumming, neutralization, water wash, bleaching, and deodorization process. Finally, citric acid has been used specifically in all treatments and control experiment. Mitigation approaches demonstrated by Ramli et al. (2020), Sim et al. (2020), and others often use phosphoric acid to degum palm oil. Every country has their own regulations and list of additives that can be used to produce organic food products. In The Netherlands, phosphoric acid is not allowed to be used in the production or organic food products. Therefore, citric acid is often used if Dutch refineries want to produce organic certified vegetable oils. Refineries in The Netherlands, but not limited to, can adopt our method of chemical refining when the production of an organic vegetable oil is desired.

The main difference between this treatment (Treatment D) and the alkali neutralization treatment (treatment C) is the deodorization temperature. Due to the lower deodorization temperature, GE formation can be kept to a minimum. Additionally, the alkali neutralization step neutralized the oil acidity which created a less favorable formation condition for 2- and 3-MCPDE to be formed as this theory was demonstrated by Šmidrkal et al. (2016). The subsequent washing step also helps with the removal of potential polar precursor elements such as chlorine salts. More investigation is however required to lower the concentrations of 2-, 3-MCPDE, and GE even more and to pinpoint other important precursors. Furthermore, GE concentration spikes were not observed during the entire refining process. Previous treatments, especially when lye was used, had a fair amount of trapped water in the oil and resulted in high GE concentrations with the combination of high deodorization temperatures. The final GE concentration in Treatment D remained low (0.99 mg/kg), even though the water content in the ‘post neutralization’ and ’wash’ sample reached 0.72% which is higher than what was observed in post-degumming water wash (Treatment B), but lower than those with alkali neutralization (Treatment C).

Looking at the FFA concentration and oil color, chemical refining can produce oil with low FFA (0.48 %) and 6.0 R / 61.0 Y in color. Compared to the single physical refining control (0.73 % FFA & 3.1 R / 31.0 Y color), the chemically refined oil has almost twice as low FFA and slightly more orange color to it, but this color difference is marginal. With these FFA and oil color results, it can be concluded that chemical refining is able to perform similarly as physical refining. Furthermore, there were no perceptible differences in the odor of the final chemically refined oil when compared to the final product of the physically refined oil. These results were only surpassed by Treatment B with 0.04 % FFA and 4.1 R / 40.0 Y color. However, the high GE concentration is undesirable and does not out-weigh the benefits of having a lower FFA concentration.

Using the chemical refining process, the final GE concentration was just below the European Commission’s ML of 1.00 mg/kg. Concerning 3-MCPDE, the observed final level of 0.78 mg/kg was well below the new ML in the EU. These new limits were recently changed (over the course of these treatments) and now also include unbound 3-MCPD. But as we focused primarily on the mitigation of bound 2-, 3-MCPDE, and GE in our study, future research should be conducted to evaluate (i) if unbound 3-MCPD is present in CPO at the start, (ii) if during the treatments new unbound 3-MCPD is generated and if this can be mitigated, and (iii) how the oil loss can be reduced.

The combination of lower deodorization temperature and a neutralization step seems to be the key to reduce 2-, 3-MCPDE, and GE simultaneously. Therefore, chemical refining could also potentially mitigate the formation of GE that requires higher formation temperatures than 2- and 3-MCPD esters.

4. Conclusions

Mitigating 2-, 3-MCPDE, and GE simultaneously would be a cost and time efficient solution for oil refineries. However, differences in the formation pathway of 2- and 3-MCPDE and that of GE hinders the development of effective mitigation strategies for these three contaminants at the same time. The aim of our study with its four experimental treatments was to develop a mitigation strategy which addresses simultaneously the mitigation of 2-, 3-MCPDE, and GE. The intrinsic effects of water washing at different moments during the refining process, acid degumming with and without neutralization with a lye, and the effect of the deodorization temperature were explored. The most successful treatment was the chemical refining, where a combination of acid degumming, lye neutralization, water washing, and mild deodorization temperatures were able to significantly mitigate the formation of 2-, 3-MCPDE, and GE simultaneously. However, we acknowledge that not all the phenomena that were observed in the pilot plan treatments can be underpinned by the existing literature. Nevertheless, this approach holds good promise for a procedure able to meet the stricter EU safety regulation now in place.

Author contributions

S.B. Oey proposed the experimental design, analyzed the samples, compiled data in tables and graphs, drafted the manuscript, and corrected comments. H.J. van der Fels-Klerx, V. Fogliano, and S.P.J. van Leeuwen contributed to scoping the study, maturing the experimental design, reviewing draft manuscript versions, and providing editorial corrections.

CRediT authorship contribution statement

Sergio B. Oey: Methodology, Investigation, Writing – original draft, Visualization. **H.J. van der Fels-Klerx:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Vincenzo Fogliano:** Conceptualization, Writing – review & editing, Supervision. **Stefan P.J. van Leeuwen:** Conceptualization, Validation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

Figs. 5–9.

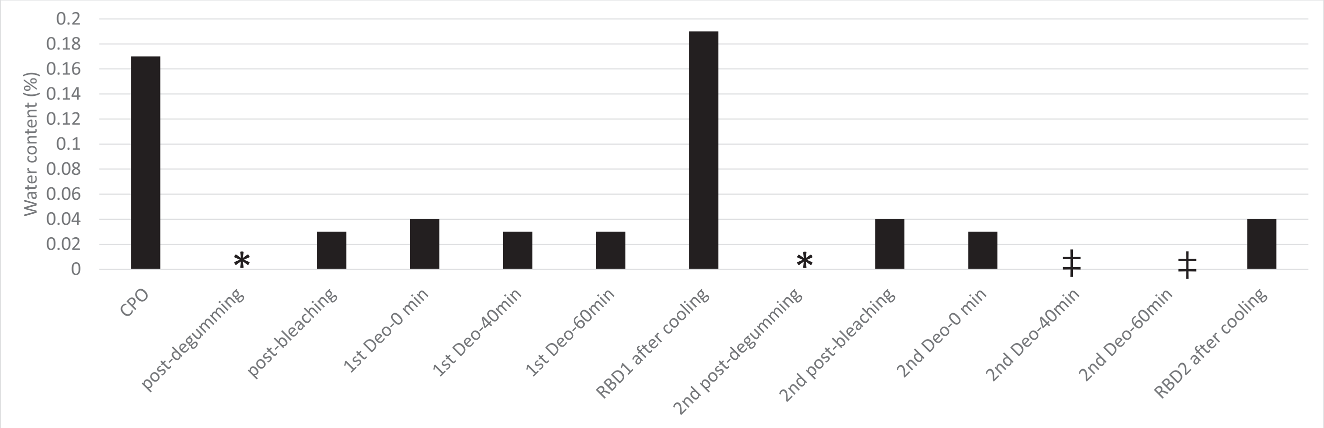

Fig. 5. Results of the water content in the collected samples during the Control Treatment. The water content in each sample was determined with the Karl-Fisher method and are expressed in percentage. The water content of the samples marked with (‡) were not determined. The samples marked with (*) were not collected.

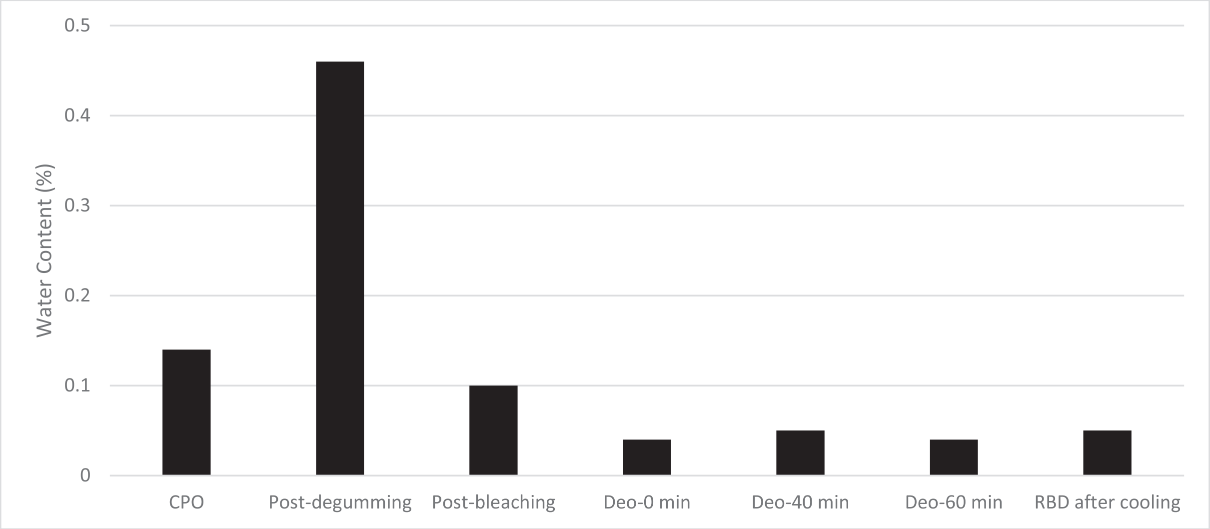

Fig. 6. Results of the water content in the collected samples during Treatment A – pre-refining water wash. The water content in each sample was determined with the Karl-Fisher method and are expressed in percentage.

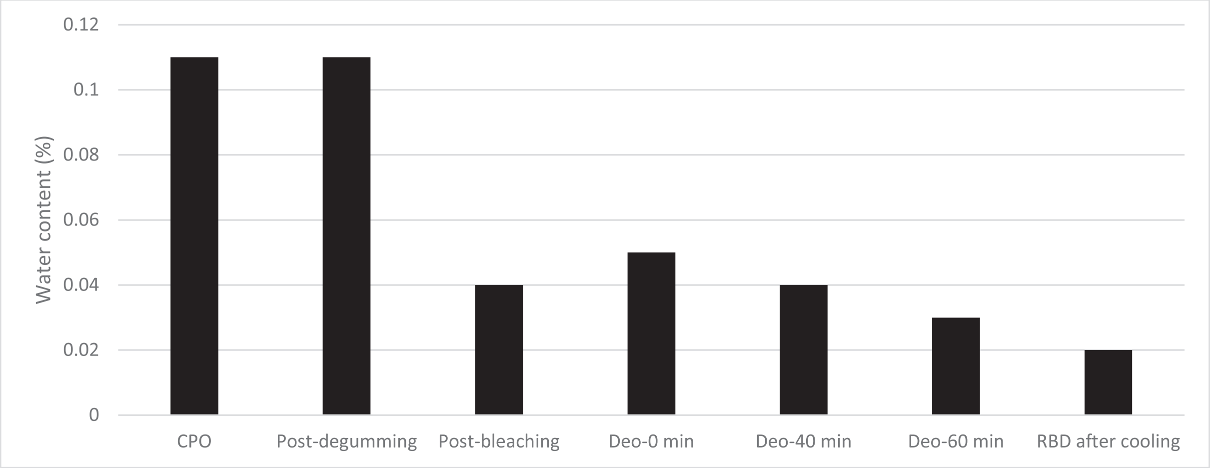

Fig. 7. Results of the water content in the collected samples during Treatment B – post-degumming water wash. The water content in each sample was determined with the Karl-Fisher method and are expressed in percentage.

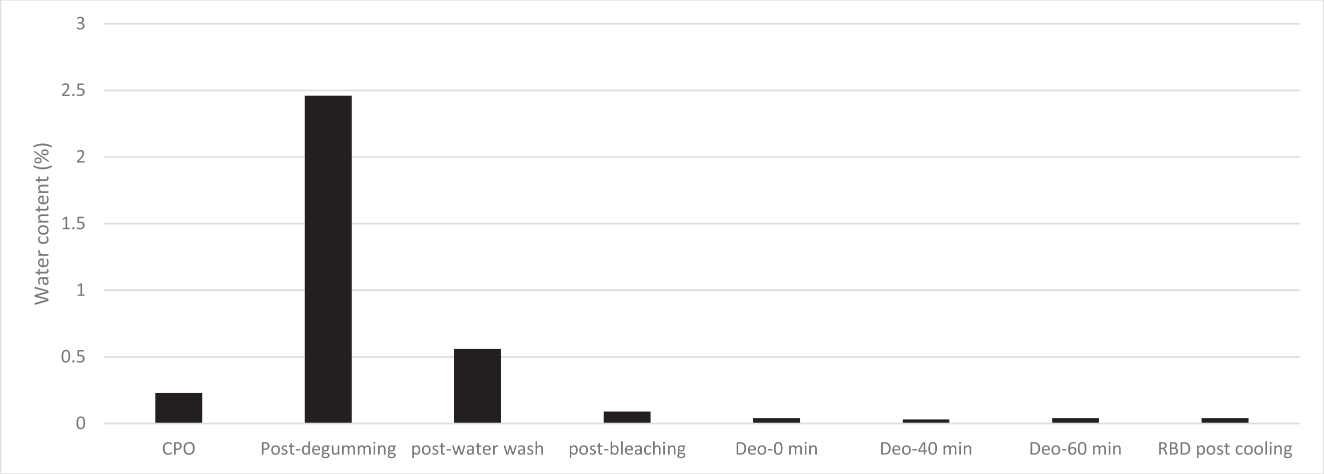

Fig. 8. Results of the water content in the collected samples during Treatment C – alkali neutralization. The water content in each sample was determined with the Karl-Fisher method and are expressed in percentage.

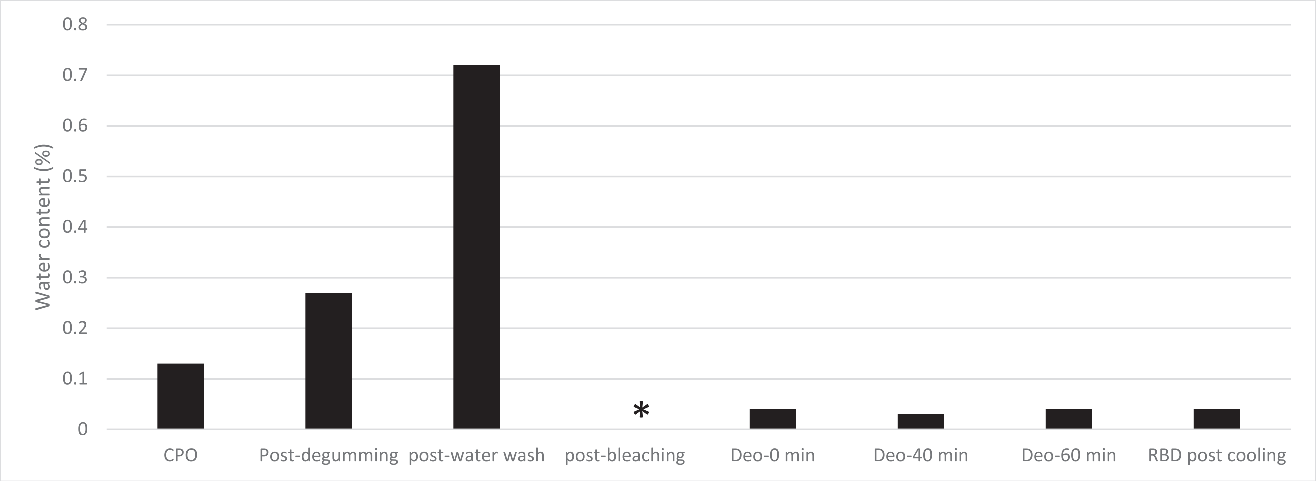

Fig. 9. Results of the water content in the collected samples during Treatment D – 'full' chemical refining. The water content in each sample was determined with the Karl-Fisher method and are expressed in percentage. The samples marked with (*) were not collected.

Appendix B. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111137.

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